

AMENDMENTS TO THE CLAIMS

1. (Original) A method of producing plants from microspores comprising:
 - (a) purifying microspores at a developmental stage amenable to androgenic induction;
 - (b) subjecting said microspores to nutrient stress to obtain stressed microspores;
 - (c) contacting said microspores with an amount of a sporophytic development inducer effective to induce sporophytic development, said contacting step occurring before, during, after, or overlapping with any portion of said nutrient stress step; and
 - (d) culturing said isolated microspores with at least one live plant ovary or with an aliquot of plant ovary conditioned medium.
2. (Original) The method of Claim 1 wherein the purified microspores are prepared by a method comprising the steps of
 - (a) selecting plant material that comprises reproductive organs containing microspores at a developmental stage that is amenable to androgenic induction;
 - (b) breaking down the selected plant material to release the microspores;
 - (c) filtering the broken down material through a screen having a mesh size large enough to permit the passage of the microspores but small enough to prevent the passage of unwanted material, said filtering step yielding a filtrate containing microspores; and
 - (d) applying the filtrate containing microspores to a screen having a mesh size that is small enough to prevent the passage of the microspores but which is large enough to permit the passage of liquid and particles smaller than the microspores.
3. (Original) The method of Claim 1 wherein the purified microspores are in the mid uninucleate to early binucleate stage of development.

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4. (Original) The method of Claim 1 wherein the purified microspores are subjected to nutrient stress by incubating the purified microspores in NPB-A medium.

5. (Original) The method of Claim 1 wherein the purified microspores are subjected to nutrient stress for a period of from 0.5 hours to 120 hours.

6. (Original) The method of Claim 1 wherein the purified microspores are subjected to nutrient stress for a period of from 1 hour to 96 hours.

7. (Original) The method of Claim 1 wherein the purified microspores are subjected to nutrient stress for a period of from 24 hours to 48 hours.

8. (Original) The method of Claim 1, wherein the purified microspores are subjected to nutrient stress by incubation in NPB-A medium for a period of from 24 hours to 48 hours.

9. (Original) The method of Claim 1, further comprising the step of subjecting the purified microspores to temperature stress, said temperature stress step occurring during or overlapping with any portion of said nutrient stress step.

10. (Original) The method of Claim 9, wherein said temperature stress step is effected by incubating the purified microspores at a temperature of from 4°C to 40°C for a period of up to 96 hours.

11. (Original) The method of Claim 9, wherein said temperature stress step is effected by incubating the purified microspores at a temperature of from 28°C to 35°C for a period of from 0.5 hour to 24 hours.

12. (Original) The method of Claim 9, wherein said temperature stress step is effected by incubating the purified microspores at a temperature of from 28°C to 35°C for a period of from 1 hour to 6 hours.

13. (Original) The method of Claim 1, wherein after the nutrient stress step the microspores are incubated at 27°C in the dark for a period of up to 48 hours.

14. (Original) The method of Claim 1 wherein said sporophytic development inducer is selected from the group consisting of 2-aminonicotinic acid; 2-chloronicotinic acid; 6-chloronicotinic acid; 2-chloroethyl-phosphonic acid; 2-hydroxynicotinic acid; 6-hydroxynicotinic acid; 3-hydroxypicolinic acid; Benzotriazole; 2-hydroxyproline; 2,2'-dipyridil; 2,4-pyridine dicarboxylic acid monohydrate; 2-hydroxypyridine; 2,3-dihydroxypyridine; 2,4-dihydroxypyrimidine-5-carboxylic acid; 2,4-dihydroxypyrimidine-5-carboxylic acid hydrate; 2-hydroxypyrimidine hydrate; 2,4,5-trihydroxypyrimidine; 2,4,6-trichloropyrimidine; 2-hydroxy-4-methyl pyrimidine hydrochloride; 4-hydroxypyrazolo-3,4,d-pyrimidine; quinaldic acid; violuric acid monohydrate; thymine; xanthine; salicylic acid; sodium salicylate; salicyl aldehyde; salicyl hydrazide; 3-chlorosalicylic acid; fusaric acid; picolinic acid; butanediene monoxime; di-2-pyridyl ketone; salicin; 2,2'-dipyridil amine; 2,3,5-triiodobenzoic; 2-hydroxy pyridine-N-oxide; 2-hydroxy-3-nitropyridine; benzotriazole carboxylic acid; salicyl aldoxime; glycine; D L-histidine; penicillamine; 4-chlorosalicylic acid; 6-aminonicotinic acid; 2,3,5,6-tetrachloride 4-pyridine carboxylic acid; alpha benzoin oxime; 2,3-butadiene dioxime; isonicotinic hydrazide; cupferron; ethyl xanthic acid; 3-hydroxy benzyl alcohol; salicyl amide; salicyl anhydride; salicyl hydroxamic acid; methyl picolinic acid; 2-chloro pyridine; 2,6-pyridine carboxylic acid; 2,3-pyridine dicarboxylic acid; 2,5-pyridine dicarboxylic acid; pichloram; ammonium thiocyanate; amiben; diethyl dithiocarbamate; glyphosate; anthranilic acid; thiourea; 2,4-dichlorophenoxyacetic acid; 4-chloro anisole; 2,3-dichloroanisole; 2-(2,4)-dichlorophenoxy propionic acid; 2-(4-chlorophenoxy)-2-methyl propionic acid; 2-(para-chloro phenoxy) isobutyric acid and α,β -dichlorobutyric acid.

15. (Original) The method of Claim 14 wherein said sporophytic development inducer is selected from the group consisting of 2-hydroxynicotinic acid, 2-chloroethyl-phosphonic acid, 2-chloronicotinic acid and 2-hydroxyproline.

16. (Original) The method of Claim 15 wherein said sporophytic development inducer is 2-hydroxynicotinic acid.

17. (Original) The method of Claim 15 wherein said sporophytic development inducer is 2-chloroethyl-phosphonic acid.

18. (Original) The method of Claim 1 wherein said sporophytic development inducer is present at a concentration of from about 0.001 mg/l to about 1000 mg/l.

19. (Original) The method of Claim 1 wherein said sporophytic development inducer is present at a concentration of from about 1 mg/l to about 500 mg/l.

20. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of an auxin, said step of contacting the microspores with an effective amount of an auxin occurring before, during, after, or overlapping with any portion of said nutrient stress step.

21. (Original) The method of Claim 20 wherein said auxin is 2,4-dichlorophenoxyacetic acid.

22. (Original) The method of Claim 20 wherein said auxin is utilized at a concentration of from about 0.1 mg/l to about 25 mg/l.

23. (Original) The method of Claim 20 wherein said auxin is utilized at a concentration of from about 0.5 mg/l to about 4.0 mg/l.

24. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of a cytokinin, said step of contacting the microspores

with an effective amount of a cytokinin occurring before, during, after, or overlapping with any portion of said nutrient stress step.

25. (Original) The method of Claim 24 wherein said cytokinin is kinetin.

26. (Original) The method of Claim 24 wherein said cytokinin is benzaminopurine.

27. (Original) The method of Claim 24 wherein said cytokinin is utilized at a concentration of from about 0.1 mg/l to about 10 mg/l.

28. (Original) The method of Claim 24 wherein said cytokinin is utilized at a concentration of from about 0.5 mg/l to about 2.0 mg/l.

29. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of a gibberellin, said step of contacting the microspores with an effective amount of a gibberellin occurring before, during, after, or overlapping with any portion of said nutrient stress step.

30. (Original) The method of Claim 29 wherein said gibberellin is utilized at a concentration of from about 0.01 mg/l to about 20 mg/l.

31. (Original) The method of Claim 29 wherein said gibberellin is utilized at a concentration of from about 0.2 mg/l to about 4.0 mg/l.

32. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of a cell spindle inhibiting agent, said step of contacting the microspores with an effective amount of a cell spindle inhibiting agent occurring before, during, after, or overlapping with any portion of said nutrient stress step.

33. (Original) The method of Claim 32 wherein said cell spindle inhibiting agent is utilized at a concentration of from about 1 μ M to about 200 μ M.

34. (Original) The method of Claim 32 wherein said cell spindle inhibiting agent is pronamide.

35. (Original) The method of Claim 1 wherein said culturing step utilizes a liquid nutrient suspension medium selected from the group consisting of medium NPB98 and NPB-99.

36. (Original) The method of Claim 1 wherein said culturing step utilizes at least one live ovary obtained from a plant variety selected from the group consisting of any wheat variety and barley variety Igri.

37. (Original) The method of Claim 1 wherein said culturing step utilizes ovary-conditioned medium.

38. (Original) The method of Claim 1 further comprising the step of filtering said cultured microspores through a filter having a pore size of 50:μm and plating said filtered microspores onto a nutrient medium, said filtration step occurring during culture of said microspores with at least one live plant ovary or with an aliquot of plant ovary conditioned medium.

39. (Original) The method of Claim 38, wherein said filtration occurs between 7 days and 10 days after the beginning of culture of said microspores with at least one live plant ovary or with an aliquot of plant ovary conditioned medium.

40. (Original) The method of Claim 1 further comprising the step of genetically transforming said microspores.

41. (Original) The method of Claim 40 wherein said genetic transformation step utilizes biolistic gene transfer.

42. (Original) The method of Claim 40 wherein said genetic transformation step utilizes electroporation of plasmolyzed microspores.

43. (Canceled)

44. (Original) The method of Claim 1, wherein microspores are wheat microspores obtained from one or more wheat spikes and at least 5000 plants are obtained per wheat spike.

45. (Original) A method of producing plants from microspores comprising:
- (a) purifying microspores at a developmental stage amenable to androgenic induction;
 - (b) subjecting said purified microspores to temperature stress and nutrient stress to obtain stressed microspores;
 - (c) contacting said microspores with an effective amount of an auxin, an effective amount of a cell spindle inhibiting agent and an effective amount of a sporophytic development inducer, said contacting step occurring before, during, after, or overlapping with any portion of said temperature and nutrient stress step; and
 - (d) culturing said isolated microspores with at least one live plant ovary or with an aliquot of plant ovary conditioned medium.